

Exploration in a T-Maze by the Crayfish *Cherax destructor* Suggests Bilateral Comparison of Antennal Tactile Information

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Abstract. Many crayfish species inhabit murky waters or have a crepuscular lifestyle, which forces them to rely on chemical and mechanical information rather than visual input. Information on how they use one form of mechanical information—tactile cues—to explore their local environment is limited. We observed the exploratory behavior of the crayfish *Cherax destructor* in a T-maze under red light. Animals moved forward along the long arm of the maze and then moved equally in one of two available directions. The arm chosen by one crayfish did not affect that selected by a second crayfish tested immediately after in an unwashed maze. Previous experience in the maze also did not affect the choice. We found, however, that crayfish with one antenna denervated or splinted back to the carapace turned more often toward the unaltered side. Our data support the hypothesis that crayfish bilaterally compare information from their antennae.

Introduction

Crayfish are found in a variety of habitats including springs, ephemeral lakes, creeks, rivers, and alpine and subtropical streams (Merrick, 1991; Lawrence and Jones, 2002). Many species are crepuscular and make regular excursions from their shelters in the first few hours after dark and before dawn (Page and Larimer, 1972; Reynolds, 1980; Corotto and O'Brien, 2002). As a result, these animals rely heavily upon nonvisual stimuli such as chemical and mechanical, including tactile, cues for moving about

and orienting (Basil and Sandeman, 2000). The major appendages for these sensory tasks are the antennules and antennae. Although these appendages are both chemosensory and mechanosensory, the antennules are the primary olfactory chemosensory organs (Cate and Derby, 2002; Grasso and Basil, 2002), while the antennae respond primarily to mechanosensory stimuli (Bush and Laverack, 1982). Each antenna consists of five basal segments and a long segmented flagellum. In some species, the antennae are as long as the body and extremely flexible (Sandeman, 1985, 1989; Zeil *et al.*, 1985). These attributes assist crayfish to locate the position of objects by using information from receptors on the flagellum (Zeil *et al.*, 1985; Sandeman, 1989).

The physiological evidence that the antennae are important in tactile responses is substantial (Bush and Laverack, 1982). Proprioceptive organs at the base of the flagellum monitor antennal movement and position (Bush and Laverack, 1982; Mellon, 2000). Changes in tactile or hydrodynamic stimulation of sensilla on the flagellum assist to determine the direction of a stimulus (Masters *et al.*, 1982). This detailed information allows the animal to determine the type of object and the distance to it (Zeil *et al.*, 1985; Sandeman and Varju, 1988). Mechanical, including tactile, input from the antennae is therefore likely to be important for navigation and exploration of terrain in the wild. In spite of this, behavioral evidence on the use of antennae during exploration of novel environments is limited (Basil and Sandeman, 2000), and there is no information on how crayfish explore or navigate in confined spaces. In streams, crayfish are often exposed to habitats that contain crevices and wooded debris. These and other features restrict or impede movement and force animals to make decisions about moving over or around obstacles.

This study investigates the exploratory behavior of the

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crayfish *Cherax destructor* (Clark 1936) in a restricted space. We used an experimental choice apparatus based on a traditional Y-maze to mimic a simple exploratory decision that crayfish might make in their natural habitat. In the maze, we compared the effects of crayfish scent, memory, and removal of sensory input from the antennae with the normal exploratory behavior of control animals.

Materials and Methods

Animals

Crayfish, *Cherax destructor*, of 6–10 cm rostrum to tailfan, were obtained from a commercial supplier and maintained in fiberglass aquaria (120 × 50 × 20 cm) at 18 °C ± 1 °C and an artificial 12 h/12 h light/dark cycle. Animals were fed pellets *ad libitum*.

Apparatus

A T-maze was constructed from PVC pipe (diameter 10 cm). An entrance arm (120 cm) and two side arms (55 cm each) were joined with a T-joint, and each arm was capped to make the maze watertight (Fig. 1). A 3-cm-wide cut was made along the top of each arm to allow us to view the crayfish. After each trial, the maze was cleaned twice with a high-pressure hose and refilled with tap water. Trials commenced 30 min after the onset of the dark period of the artificial light/dark cycle and continued for 3–6 h, a period

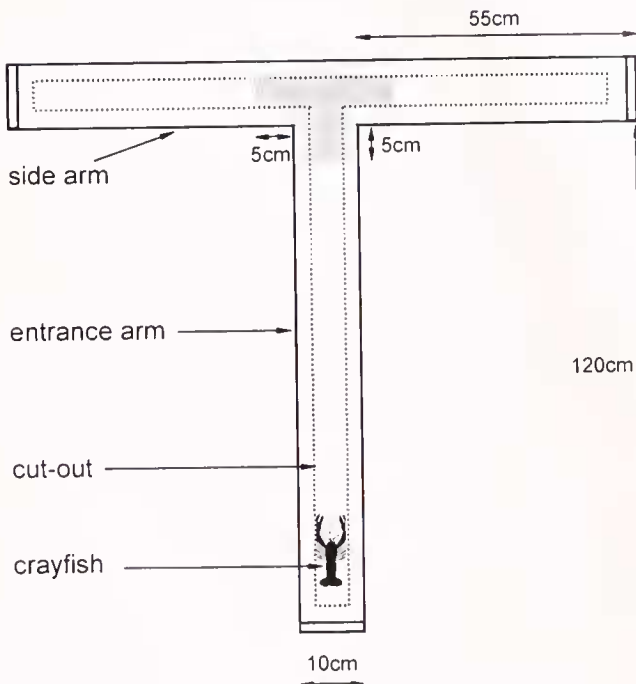


Figure 1. The T-Maze. Crayfish were placed in the bottom of the entrance arm and viewed through the cut-out as they proceeded to the junction (shaded area) and into a side arm.

when other species of crayfish are most active (Page and Larimer, 1972). A 15-W light equipped with a red darkroom filter (Ilford) was suspended 1.8 m above the maze, producing 0.8 ± 0.1 lux of illumination at maze level. This light enabled the animals to be viewed during trials but was relatively undetectable to them (Cronin and Goldsmith, 1982). To verify that the light was too dim for crayfish vision, we placed 10 crayfish, one at a time, in a plastic container (20 × 20 × 10 cm) and waved our hands back and forth 1 m above each animal. None of the 10 crayfish responded to the movement as the animals do in daylight and as described for other species (Beall *et al.*, 1990).

Trials

Crayfish were held in a plastic container (20 × 20 × 10 cm) for 2–4 min (except where otherwise stated) and then placed in the base of the maze. They proceeded along the maze and, at the junction, moved into one of the two side arms. A crayfish was deemed to have entered the junction when its rostrum crossed a line 5 cm before the top of the entrance arm and to have exited the junction after crossing a line 5 cm into either side arm. When an animal exited the junction, it was recorded as having turned either left or right. Preliminary observations suggested that this was a reliable indicator of arm choice because crayfish seldom turned back. The time taken to reach the junction and the time spent in the junction were recorded with a stopwatch for each trial.

Crayfish were randomly divided into five groups: (1) 20 crayfish to test arm choice and general exploratory behavior; (2) 20 to test whether response is influenced by conspecific scent; (3) 10 that were tested 10 times each to determine whether arm choice is influenced by experience in the maze; (4) 80 to test whether removal of sensory input from the antennae affects exploratory behavior; and (5) 20 to test whether splinting back of one antenna had the same effect on exploratory behavior as removal of sensory input.

Denervation of antennal flagellum

A small flap, about 1 × 1 mm, was cut in the dorsal cuticle of the flagellum between the 1st and 10th annuli. A miniature wire hook was inserted into the flap, twisted around, and pulled out to sever the nerve. The antenna was not denervated in the basal segments, because proprioceptors between the articulations of these joints monitor antenna movement and denervation of these structures might disrupt the animal's thigmotactic behavior (Hartman and Austin, 1972; Sandeman, 1985; Basil and Sandeman, 2000). The level of the denervation was thus selected to remove the distal sensory information it supplies to the animal without disrupting the proximal proprioceptors. Crayfish were sacrificed after experiments and dissected to confirm the status of the denervation.

To control for any surgical effects, sham denervations were performed identically, except that the nerves were neither hooked nor severed, and care was taken to avoid severe bleeding in case this could produce clotting and damage the nerve.

Splinting of antennal flagellum

The antennal flagellum was splinted to the top of the crayfish's carapace. A small plastic tube (6–8 mm length) was attached with cyanoacrylate adhesive to the carapace centrally at the fusion between head and thorax. Crayfish were then given 24 h to adapt before experimentation. Thirty minutes prior to a trial the crayfish was placed in a plastic container (20 × 20 × 10 cm), and its left or right antenna was splinted by feeding it through the plastic tube on the carapace. The flagellum was held in place with a small amount of adhesive at the distal end of the tube to secure it to the animal's back. The splint was checked after the experiment to confirm that it was still in place.

Analysis

General exploratory behavior. Arm choice was scored as either left or right and analyzed with a chi-square test. A Yate's correction was applied because there were only two alternatives in the maze (Fowler *et al.*, 1998).

Influence of scent. A runs test for dichotomized data was used to compare right and left choices for randomness (Sokal and Rohlf, 1995). This test determines whether events occur in a random sequence or whether the probability of a given event is a function of the outcome of a previous event.

Influence of memory. To check for any patterns in each trial, time taken to walk up the entrance arm and time spent in the junction were analyzed for each crayfish with a runs test above and below the median.

Removal of sensory input. Arm choice was compared with a Yate's corrected chi-square test for each group of left and right denervated animals. Time taken to walk up the entrance arm of the maze and time spent in the junction were pooled for denervated and sham groups, log transformed to normalize data, and compared with a Student's *t*-test using Systat 10.2.

Results

General exploratory behavior

When placed in the T-maze, crayfish spread their antennae to touch the sides before starting to walk. They then walked up the middle of the tube with both antennae held out in front, touching the walls on either side of the maze,

to guide themselves (thigmotaxis). The tubular arms of the maze meant that animals walked roughly in the center with no apparent deviation. At the junction, they scanned left and right with their antennae before turning into one arm of the maze. Animals entered the side arms without preference for a particular direction: 11 animals turned right and 9 turned left ($n = 20$, $df = 1$, $\chi^2 = 0.25$, $P = 0.617$).

Influence of conspecific scent

Twenty crayfish were tested in the maze when it was not washed between trials. These animals were selected randomly from a densely populated communal holding tank (≈ 60 animals/m²) to minimize differences in social status between individuals and to ensure some group familiarity. The result of a trial was recorded as a choice between the same or different arm chosen by the animal in the immediately preceding trial. A "dummy" crayfish was run in the maze at the start of the experiment but excluded from the analysis. If a crayfish failed to walk through the junction and returned down the entrance arm, it was excluded and another dummy crayfish was used before the experiment continued. Once a crayfish had entered a side arm, a mesh fence was inserted by hand into that arm, between the junction and the animal, to prevent the crayfish from turning back during capture. The crayfish was then removed with a net. In these experiments, 9 crayfish went in the same direction as the previous animal and 11 went into the opposite arm (runs test, $P > 0.05$). This result suggests that either no scent was left by other crayfish in the maze or there was no preference for animals to follow the preceding individual.

Influence of memory

Ten crayfish were each tested in 10 consecutive trials. Between all trials, the maze was washed and animals were rested for 2–4 min in the plastic container. There was no significant difference in arm choice for any crayfish in its 10 repeated trials (runs test, all $P > 0.05$; see Fig. 2). Seven crayfish showed random behavior in time taken to reach the junction (runs test, $P > 0.05$), whereas three showed a significant pattern over their 10 trials (runs test, $P < 0.05$). Nine of the crayfish showed random behavior for time spent in the junction (runs test, $P > 0.05$), and only one displayed a significant pattern (runs test, $P < 0.05$).

Effect of the removal of antennal sensory information

Removal of sensory input from one of the antenna had a pronounced effect on behavior and arm choice. Crayfish with a denervated flagellum often trailed that appendage behind when they walked. They walked down the center of the maze with the intact antenna touching the side, similar to the intact animals. These animals turned toward the side of the intact antenna. The response was consistent whether

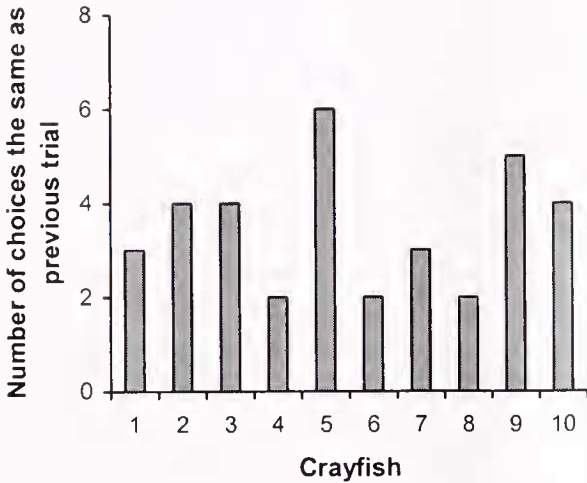


Figure 2. The number of choices out of 9 that each crayfish made that were the same as in its previous trial in the maze.

the right ($n = 20$) or left ($n = 20$) flagellum was denervated. Of the 20 animals with the right antenna intact, 15 turned into the right arm ($df = 1$, $\chi^2 = 5.05$, $P = 0.025$); of the 20 with left antenna intact, 16 turned left and 4 right ($df = 1$, $\chi^2 = 7.25$, $P = 0.007$).

The sham-operated animals showed no preference for either side arm. Crayfish with a sham-denervated left flagellum turned left 11 times and right 9 ($n = 20$, $df = 1$, $\chi^2 = 0.25$, $P = 0.617$); those with the right flagellum sham-denervated turned left and right 10 times each ($n = 20$, $df = 1$, $\chi^2 = 0.05$, $P = 0.823$). There was no noticeable difference in behavior of this group compared to unoperated crayfish, and we concluded that the surgery itself did not affect the behavior.

Because arm choice was not different between the left and right groups in each category of surgery, the data on time in the maze were pooled to compare the denervated flagellum group with the sham group. Time taken to walk to the junction was not different between the two groups ($n = 80$, $df = 78$, $t = -0.312$, $P = 0.756$; Fig. 3A). Crayfish with a denervated flagellum spent less time in the junction than sham-operated animals ($n = 80$, $df = 78$, $t = 3.102$, $P = 0.003$; Fig. 3B).

Effect of immobilizing one antenna on exploratory behavior

Splinting either the left or right antenna behind the crayfish's head had a pronounced effect on arm choice in the maze. These crayfish, like the denervated group, turned toward the side of the unmanipulated antenna: 16 crayfish turned toward the side of the free antenna and 4 to the splinted side ($n = 20$, $df = 1$, $\chi^2 = 6.050$, $P = 0.025$).

Discussion

When individuals of *Cherax destructor* were placed in a T-maze, they used thigmotactic information to navigate. This behavior was used to test directional responses of animals under conditions that may reflect situations in the wild. The antennae were important in exploration of the maze. When tactile information was removed from one antenna by denervation or was altered by splinting one antenna back, the crayfish explored the maze on the side of the unaltered appendage.

Crayfish may have moved to the maze arm of the side of the intact antenna because they could detect surrounding stimuli in that arm. This may signify a tendency to move toward a safer, known sensory environment. Basil and Sandeman (2000) observed that crayfish follow walls in preference to open space and closely hug objects that jut out

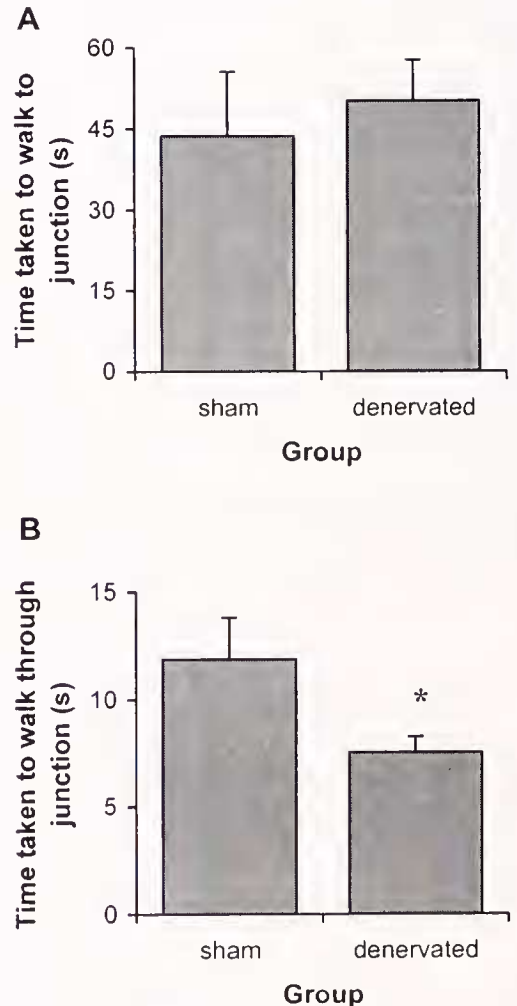


Figure 3. Mean time (\pm SEM) taken to reach the junction (A) and spent in the junction (B) for crayfish with one denervated flagellum and for sham-operated crayfish. Asterisk (*) indicates a significant difference.

from a straight wall. In those situations, animals compared known tactile information from the wall with no information from the water on the other side, which supports the idea that crayfish prefer to explore identified environments. This is also supported in our experiments by the finding that denervated animals make a decision in the junction of the maze faster than intact animals.

Our data suggest that crayfish compare tactile input from the two antennae. The finding that both denervated and splinted crayfish turned toward their unaltered side suggests that the response was not some artifact due to the artificial removal of sensory information from the denervation procedure, but rather that they were comparing inputs from both antennae. When both antennae detected tactile cues in the maze, animals explored randomly; however, when input was removed from one appendage, turns were biased toward the side from which information was still arriving.

It has been proposed that crayfish and other decapod crustaceans use bilateral comparison between the antennae or antennules to orientate toward chemical cues (lobsters: Reeder and Ache, 1980; Atema, 1996; Beglane *et al.*, 1997; crayfish: Kraus-Epley and Moore, 2002; review: Grasso and Basil, 2002). When considered in conjunction with these studies, our results suggest subtle differences between the way chemical information and tactile information are used for this comparison. We observed that animals moved faster in the junction when tactile input was reduced, whereas there is evidence to suggest that selective absence of chemical input produces slower movement (Kraus-Epley and Moore, 2002).

Crayfish encounter a variety of scents in the wild. Some decapod crustaceans are sensitive to chemical cues at nanomolar and picomolar concentrations (Derby and Atema, 1982). Crayfish release urine in bursts (Breithaupt and Eger, 2002), and thus animals may be able to detect other individuals in the maze. It is known that crayfish and shrimp are attracted to the scent of familiar conspecifics (Ward *et al.*, 2004; Crook *et al.*, 2004). In our experiment, we administered scent in a way that crayfish may encounter it in the wild; however, this was not sufficient to affect the direction explored by an individual. This may have been because they could detect the residual scent of previous trials in both arms, or because no scent trail was laid in the short time that animals were in the maze. Given that crayfish were maintained in high-density communal tanks, individuals were likely to be familiar with each other in our experiments, or at least to have recognized that they were from the same cohort. Testing with scents added in a more guaranteed manner, albeit also more artificial, may reveal a different result.

When compared to other studies, our research suggests that the learning mechanisms of crayfish may differ depending on the topography of the environment. Basil and Sandeman (2000) found that crayfish learned the environment of

a large open test arena and moved more rapidly through it as it became more familiar. If crayfish had remembered their previous choice in the T-maze, one would predict that they would make a choice more quickly in subsequent trials. However, our time data suggest that this is unlikely. Furthermore, we would expect arm choice to be the same. It remains possible that crayfish remembered the maze, but that the memory did not result in a response to move to a familiar environment. The result might be different when a chosen direction is reinforced by some resource such as food or shelter. In such situations, animals would be expected to turn in the direction that rewards a particular choice.

The extent that vision interacts with the tactile system is unclear. Antennal movement appears to be different between sighted and blinded crayfish (Zeil *et al.*, 1985). The combination of additional sensory input and tactile information may influence exploration in spaces such as the T-maze or in the wild, but our darkened conditions eliminated this factor. This situation has a parallel in haptic perception in humans. The haptic sense refers to the movement of limbs to gather information about their position in space and about objects they encounter by touch. The crayfish system presents a model that may help explain how visually impaired people gain conscious knowledge of position (Zeil *et al.*, 1985).

This study highlights the importance of the antennae to crayfish performing directional responses in restricted spaces. The role of the antennae may have implications for crayfish living in a dynamic environment where all senses may not always be available. It remains to be determined whether the effect of denervation is temporary or persists until the crayfish grows a new antenna.

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